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A RADIOACTIVE TRACER STUDY OF FOOD UPTAKE BY *PINNOTHERES MACULATUS* IN MOLLUSCAN HOSTS

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The Pinnotheridae, pea crabs, is a group of decapod crustaceans adapted for living with other marine animals. Pearce (1962) discussed adaptations of these crabs for symbiotic existence, and Gotto (1969) listed bivalves, gastropods, sea slugs, chitons, polychaetes, echinoderms, burrowing crustaceans and sea squirts as known hosts.

Associations of most pinnotherids with their hosts have not been defined. Many have long been recognized as commensals, and some have been labeled parasitic (Cheng, 1967; Gotto, 1969). *Pinnotheres ostreum* (Say), for example, is known to cause gill and palp damage to *Crassostrea virginica* (Gmelin) and reduce growth and reproduction of the oyster (Overcash, 1946; Sandoz and Hopkins, 1947; Flower and McDermott, 1952; Haven, 1958; Christensen and McDermott, 1958). McDermott (1962) found that both *P. ostreum* and *Pinnotheres maculatus* Say caused similar damage in *Mytilus edulis* Linné. *Fabia subquadrata* Dana was observed to damage gills and cause cyst-like anomalies in mantle tissues of *Modiolus modiolus* Linné (Pearce, 1966a) and *Pinnotheres* sp. damaged gill, mantle and gonad of *Meretrix casta* (Chemnitz) (Silas and Alagarswami, 1967).

The mode of feeding of some pinnotherids has been investigated. Pearce (1966b) noticed that *Pinnixa fabra* (Dana) caused damage to its host clam, *Tresus capax* (Gould), and found that the crab ingested food which would otherwise be available to the clam. Stealing of food which a host has concentrated into food strings has also been reported for *Pinnotheres pisum* (Pennant) (Coupin, 1894; Orton, 1921), *Pinnotheres concharum* (MacGinitie and MacGinitie, 1968) and *P. ostreum* (Stauber, 1945). Campbell (1969) found plant pigments in the gut of *P. pisum* which indicated ingestion of algae. Orton (1921) and Gotto (1969) suggested that some crabs may also filter food from water currents created by hosts. All of these studies are descriptive and no experimental quantification of crab-host associations are known except that adult female *P. maculatus* stunted growth of bay scallops, *Argopecten irradians concentricus* (Say), grown in cages (Kruczynski, 1972).

Radioisotope techniques are common tools to study physiological processes, but few studies are known employing these methods to quantify trophic relationships in natural ecosystems. Poole (1974) discussed some methods of measuring ingestion using radioactive tracers and reviewed research which quantified components of some terrestrial and stream ecosystems by these methods. Smith, Muscatine and Lewis (1969) reviewed quantification of carbohydrate movement using ^{14}C from autotrophs to heterotrophs in parasitic and mutualistic symbiosis. Pardy and Muscatine (1973) used labeled green algae to quantify uptake of symbionts by *Hydra viridis*.

Phytoplankton labeled with radioisotopes has been used to measure water filtering rates of some ascidians and lamellibranchs (Jorgensen and Goldberg, 1953; Chipman and Hopkins, 1954; Rice and Smith, 1958). This present study reports use of phytoplankton labeled with ^{14}C to quantify *in situ* the association of adult female *P. maculatus* with its common hosts, the bay scallop, *A. irradians*, calico scallop, *Argopecten gibbus* (Linné), and blue mussel, *M. edulis*, under laboratory conditions.

Materials and Methods

Collection and maintenance of organisms

Feeding experiments with bay and calico scallops were conducted in North Carolina. Bay scallops were collected from Bogue Sound and calico scallops from off Cape Lookout, North Carolina. Scallops were cleaned of encrusting organisms and kept at room temperature (20–25° C) in large plastic trays containing aerated seawater. Feeding experiments with blue mussels were performed at the Marine Biological Laboratory, Woods Hole, Massachusetts. Mussels were collected from pier pilings of the Woods Hole Oceanographic Institution and kept in running seawater at room temperature (20–22° C).

Preliminary observations insured that selected diatoms were filtered and ingested by mollusc hosts. *Nitzchia closterium* was used in experiments to follow uptake of phytoplankton into scallops, crabs in scallops and crabs in finger bowls containing phytoplankton. *Thalassiosira pseudonana* was used in experiments with mussels. Cultures were grown at room temperature in half-strength Guillard's medium (Guillard and Ryther, 1962) prepared with 30‰ seawater. Radioactive carbon was added as $NaH^{14}CO_3$ before inoculation of new medium with stock culture, and cultures grew at least 7 days before used in an experiment. Assay revealed that much of the tag was found in the diatoms, although some was left in solution.

Assay of radioactivity

Radioactivity in scallop experiments was determined with a Dynacon (Nuclear Chicago, Model 6010). This instrument has been used in studying carbon fixation by aquatic plants (Dillon, 1971) and is sensitive to low amounts of β radiation. It has a counting efficiency of nearly 100% but only a few samples can be run per day. Radioactivity of CO_2 in dry, combusted samples is measured. A Packard Liquid Scintillation Spectrometer was used in mussel experiments. Samples were oxidized in a Packard Tri-Carb Automatic Sample Oxidizer. Resultant $^{14}CO_2$ was absorbed in scintillation vials. Quenching characteristics of oxidized samples are nearly constant (Faires and Parks, 1973) and no quench corrections were made. Counting efficiency of this system was nearly 80%.

Bay scallops

Background radioactivity of dried tissues of bay scallops was determined by assaying aliquots. Uptake of ^{14}C from solution was measured by filling two bowls with 1 liter of seawater and 1.6 μCi ^{14}C (2.2×10^6 cpm/μCi). A bay scallop was

placed in each bowl for 24 hours and water was aerated with breakerstones. On removal, scallops were rinsed with filtered water and dried to a constant weight at 80° C. Aliquots of scallop tissues were assayed and total radioactivity estimated.

Feeding experiments were performed with six noninfected scallops. A control bowl (no scallop) was run per pair of experimental bowls. Bowls were filled with 1 liter of water and a measured amount of radioactive culture of *N. closterium*. Cell counts were made to assure approximate equality and bowls were covered with a glass plate and aerated. A scallop was placed in each experimental bowl for 24 hours, after which contents of control and experimental bowls were centrifuged in a continuous plankton centrifuge and filtered with a Millipore filter (HA 0.45 μ). Particulate matter from the control bowl was used as an estimate of initial phytoplankton activity; that from experimental bowls represented phytoplankton that was rejected as faeces, pseudofaeces and unfiltered phytoplankton. No effort was made to separate activity in the three categories of "rejected materials."

Experimental scallops were then placed in filtered water for 24 hours, removed from the shells, dried and weighed. Contents of bowls containing scallops for the second day were recovered and the activity added to the rejected material. Aliquots of scallops and whole dried filteres were assayed and total counts per minute (cpm) per scallop estimated.

Twelve additional bay scallops were infected with an adult female crab 3 days prior to experiments. I amputated the chelae from one crab before placing it in a scallop. Crabs were kept isolated in finger bowls for at least 7 days before being placed in scallops.

A control bowl was run per pair of scallops tested and bowls were treated as above. After the second day, crabs and scallop tissues were dried, weighed and assayed.

Calico scallops

Two calico scallops, each containing its naturally occurring adult female crab, were tested as above. A control bowl was run for each.

Blue mussels

Seven mussels were selected from an area where most were known to contain an adult female crab. They were placed in bowls containing a known concentration and activity of radioactive *T. pseudonana* and allowed to feed for 24 hours. Mussels were then placed in filtered seawater for 24 hours. In one trial the chelae of an adult female crab were removed and it was placed back into a mussel which was relaxed in isosmotic $MgCl_2$ and kept in running seawater for one day before testing. One control mussel and crab was treated in the same manner except no radioactive phytoplankton was added to the dish.

Pinnotheres maculatus *in finger bowls*

Six crabs were tested to determine whether crabs can ingest phytoplankton which has not been concentrated by a host. Each trial included two experimental and one control bowl. The same amount of a culture of tagged specimens of *N.*

TABLE I

Uptake of radioactivity by adult female specimens of Pinnotheres maculatus *and molluscan hosts. Mean values given, standard deviations in parentheses.*

Species	n	Initial diatoms cells/ml × 10^3	Host cpm/mg	Crab cpm/mg	Per cent of initial radioactivity			
					Recovered	In host	In crab	Rejected
A. irradians								
No crabs	6	210	151(40)	—	81(24)	23(14)	—	58(30)
With crab	11	240	188(108)	195(130)	78(10)	40(11)	3(1)	36(19)
With clawless crab	1	810	87	0	97	35	0	62
A. gibbus								
With crab	2	150	419(7)	823(58)	85(11)	40(11)	7(2)	38(3)
M. edulis								
No crabs	2	330	16(6)	—	95(30)	24(5)	—	71(37)
With crab	5	340	18(5)	142(146)	78(9)	28(12)	2(1)	48(8)
With clawless crab	1	350	15	27	75	6	2	67
Finger bowl								
Clawed crab	5	144	—	171(130)	79(11)	—	21(8)	58(10)
Clawless crab	1	110	—	15	86	—	3	83

closterium was added to the bowls in each trial. A female crab was placed in each of the experimental bowls which were shaded with paper towels. After 24 hours, crabs were rinsed with filtered water, blotted dry, and contents of all bowls filtered. One experimental bowl contained a crab with amputated chelae.

RESULTS

Bay scallops

Background activity of bay scallops was 0.20 cpm/mg dry weight. Scallops kept in water with ^{14}C in solution became tagged and contained an estimated 3500 cpm (3 cpm/mg) probably because of physical exchange of water, incomplete washing of tissues, or both. This uptake may be a source of error in other experiments, but it was disregarded as a major factor because of the low activity for the amount of ^{14}C used.

Since the amount of radioactivity per amount of phytoplankton varied between trials in feeding experiments, comparison of actual numbers between experiments are less valuable than comparing percent recovery of initial tagged material.

In experiments with noninfected bay scallops, recovery of radioactivity varied from 42 to 100% of estimated initial activity (Table I). Total radioactivity of noninfected scallops varied from 9 to 41% and rejected material, that is faeces, pseudofaeces and uneaten diatoms, varied from 19 to 88% of the initial tag. There was much pseudofaeces in all bowls containing scallops. All scallops were radioactive and activity varied from 90 to 206 cpm/mg, indicating an accumulation of ^{14}C under these conditions. There was little correlation between scallop dry weight and per cent uptake ($r = 0.45$).

In experiments with bay scallops infected with crabs, recovery of initial radioactivity varied from 66 to 97%. Scallops and crabs rejected 12 to 72% and scallops contained 16 to 53% of the estimated initial tag. Radioactivity of scallops varied from 51 to 337 cpm/mg. Crabs contained between 1 and 6% of the initial tag and activity varied from 64 to 396 cpm/mg (Table I), indicating that clawed crabs accumulate ^{14}C under these conditions. There was little correlation between crab dry weight and percent uptake ($r = 0.39$). The clawless crab contained no radioactivity above background.

Calico scallops

Calico scallops became tagged with 414 and 424 cpm/mg dry weight. Crabs contained 6 and 9% of initial tag and had an activity of 782 and 864 cpm/mg.

Blue mussels

Control mussel and crab contained no radioactivity above background. Between 65 and 118% of initial radioactivity was recovered. Mussels contained from 6 to 44% of the initial radioactivity on diatoms and had an activity of between 5 and 24 cpm/mg. There was little correlation between mussel weight and per cent uptake ($r = 0.46$). Five of the mussels harbored a female crab and crabs contained 24 to 355 cpm/mg. The clawless crab accumulated 27 cpm/mg. The correlation coefficient of crab uptake and dry weight was 0.23.

Pinnotheres maculatus *in finger bowls*

Clawed crabs became tagged with 59 to 396 cpm/mg. The clawless crab contained 15 cpm/mg (Table I). Between 7 and 28% of initial activity added as phytoplankton was found in clawed crabs. Gut analysis confirmed that crabs ingested diatoms.

Discussion

The morphology of adult female specimens of *P. maculatus* is specialized for existence in mollusc hosts. Males are specialized for swarming and are capable of living outside hosts. Patton (1967) observed that commensal crustaceans found in sheltered situations have a stout form, often accompanied by increased egg production, and a soft exoskeleton which could be selectively favored by not irritating hosts enough to seriously disturb food intake. I have placed adult female specimens of *P. maculatus* into noninfected bay scallops and have observed that three to five days is ample time for hosts to adapt to the presence of crabs and resume normal filtration rates (Kruczynski, 1971).

Pearce (1962) also noted considerable differences in the integument of pinnotherids and the remainder of brachyurans. Males generally have a hard exoskeleton and their importance seems restricted to copulation. Patton (1967) suggested that any mechanism which would result in noncommensal males would have a selective advantage because in some cases a single female crab can seriously affect a host. Thus, males were not used in my study because their effect on nutrition of a single host is probably small since they move from host to host.

This should be tested in future experiments. Adult female crabs, however, are virtually trapped in the mantle cavity of molluscs and have more effect on a single host. Demonstrating that female specimens of *P. maculatus* accumulate ^{14}C in scallops and mussels feeding on labeled diatoms strengthens the argument for its being the causative agent of observed stunting of bay scallops under natural and experimental conditions (Kruczynski, 1972).

Many commensal crabs rely on hosts to concentrate food into food strings. My results indicate that *P. maculatus* can also ingest *N. closterium* which has settled in finger bowls. These crabs were observed to actively pick at the bottom of the dishes and continuously clean themselves. It is still not known whether *P. maculatus* has any ability to filter their own food. Adults seem unadapted morphologically for that mode of feeding. The fact that clawless crabs in scallop, mussel and dish had low activities supports this.

Bivalves filter large volumes of water in feeding. Chipman and Hopkins (1954) followed the decrease of radioactive *N. closterium* from the medium to demonstrate that bay scallops (64 mm shell height) filtered about 15 liters per hour. They found no apparent difference in filtering rate in suspensions of phytoplankton of different concentrations and they did not measure how much phytoplankton was ingested. Rice and Smith (1958) found that *Mercenaria mercenaria* (Linné) filtered *N. closterium* in concentrations between 400 cells/ml to 170×10^3 cells/ml. Clams formed large amounts of pseudofaeces in high concentrations of algae. Perhaps the large percentage of tag found in pseudofaeces (rejected material) in my experiments is the result of using high cell concentrations. Further studies might be more useful if natural concentrations of phytoplankton were used. It is probable that effects of removal of phytoplankton by crabs from hosts is more pronounced under conditions of natural phytoplankton concentrations.

Experimental *P. maculatus* accumulated 3%, 7% and 2% of radioactivity used in bay scallop, calico scallop and mussel experiments respectively, but it is still not known what part of this activity came from ingestion of phytoplankton, faeces, pseudofaeces, molluscan tissues, or from the water. Many workers have shown that some aquatic organisms take up more radionuclides from water than from food pathways (Mauchline and Templeton, 1964; Polikarpov, 1966). This error could be reduced in future experiments by washing phytoplankton cells before adding them to experimental dishes. Washing would also reduce error resulting from continued fixation of ^{14}C by diatoms while experiments were in progress. I think that direct uptake or physical sticking of phytoplankton cells to experimental animals were not important sources of error in my experiments because of the low correlations between dry weights and percent of tag recovered. If contamination was a major factor, there would be a direct relationship between the amount of tissue and activity of experimental animals in which nearly the same amount of tag was used. This was not observed.

Other pinnotherids should be examined in a similar way to determine food uptake in hosts so that associations may be defined. Tagged hosts might be used to determine ingestion of mucus or host tissues.

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Summary

Adult, female specimens of *Pinnotheres maculatus* accumulated radioactivity when in bay and calico scallops fed *Nitzchia closterium* labeled with ^{14}C and in blue mussels fed labeled *Thalassiosira pseudonana*. Crabs accumulated radioactivity when kept in fingerbowls with labeled *N. closterium*.

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